

AMENDMENTS TO THE CLAIMS

1. (currently amended) A biochip comprising:

- a) a solid substrate having a flat top surface;
- b) a plurality of optically clear, individual, three-dimensional

hydrogel cells at least 20 μm thick attached to the flat surface of the substrate at discrete locations ~~in~~ to form an array of discrete individual three-dimensional cells protruding from said otherwise flat top surface, which hydrogel cells are formed from an isocyanate-functional prepolymer with urethane linkages; and

- c) a different binding entity immobilized within or upon various of said hydrogel cells by covalent linkage of said binding entity or an intermediate agent with reactive isocyanate groups of said hydrogel, which entity is effective to selectively hybridize to or sequester a target molecule.

2. (canceled)

3. (previously presented) The biochip of claim 1 wherein the hydrogel comprises polyethylene glycol, polypropylene glycol, or copolymers thereof having a molecular weight of about 5000.

4. (canceled)

5. (currently amended) The biochip according to claim 3, wherein the ~~three-~~
dimensional individual hydrogel ~~cell is~~ cells are between about 30 μm and about 100 μm
thick.

6. (currently amended) The biochip according to claim 1, wherein said
binding entity is directly covalently bound to and within the hydrogel cell through
reaction with the isocyanate groups.

7. (currently amended) The biochip of claim 6 1 wherein about 15% ~~or less~~
to about 5% of the reactive isocyanates in said ~~polymer of prepolymer that forms~~ said cell
have reacted ~~with~~ to immobilize said binding entities or said intermediate agents.

8. (withdrawn) The biochip of claim 1 wherein each said binding entity
comprises DNA, RNA or PNA.

9. (previously presented) The biochip of claim 1 wherein each said binding
entity comprises an immunoglobulin, an enzyme, a receptor, an enzyme inhibitor, an
enzyme substrate, or a peptide.

10. (original) The biochip of claim 9 wherein each said binding entity is
immobilized within the hydrogel through an interaction with an intermediate agent.

11. (withdrawn) The biochip of claim 1 wherein each said binding entity is a protein which is bound to a metal chelate that is immobilized in the hydrogel and constitutes said intermediate agent.

12. (withdrawn) The biochip of claim 11 wherein said protein is bound to said metal chelate through a histidine-containing polypeptide at one terminal of said protein.

13. (withdrawn) The biochip of claim 1 wherein each said binding entity is immobilized through a first intermediate agent linked to the hydrogel and a second intermediate agent linked to said first intermediate agent.

14. (withdrawn) The biochip of claim 13 wherein said first intermediate agent is an antibody and the second intermediate agent is a protein.

15-16. (canceled)

17. (previously presented) The biochip of claim 1 wherein the substrate is optically transparent and has reactive molecules on its top surface to which the hydrogel is covalently bound through some of said isocyanate groups of the polymer.

18. (currently amended) A hydrogel biochip comprising:

a) a solid substrate having a flat top surface;

- b) a plurality of optically clear, individual, three-dimensional hydrogel cells of an isocyanate-functional hydrogel at least about 20 μm thick, comprising polyethylene glycol, polypropylene glycol, or copolymers thereof having urethane linkages, bound to the top surface of said substrate at discrete locations ~~in~~ to form an array of discrete individual three-dimensional cells protruding from said otherwise flat top surface;
- c) intermediate agents immobilized within or upon said hydrogel cells by covalent binding to reactive isocyanate groups of said hydrogel; and
- d) different protein binding entities bound to said intermediate agents respectively within at least several of said hydrogel cells by interaction therewith in a manner so that said protein binding entities assume their native conformations.

19-30. (canceled)

31. (currently amended) A biochip comprising:

- a) a solid substrate having a flat top surface;
- b) a plurality of optically clear, individual, three-dimensional hydrogel cells at least about 20 μm thick attached to the surface of the substrate at discrete locations ~~in~~ to form an array of discrete individual three-dimensional cells protruding from said otherwise flat top surface, each hydrogel cell being a polymer formed from an isocyanate-functional urethane prepolymer polymer and having urethane linkages; and

c) different protein binding entities immobilized via linkage to isocyanate groups of said hydrogel within or upon different said hydrogel cells, each protein binding entity being effective to selectively hybridize to or sequester a target molecule.

32. (previously presented) The biochip of claim 31 wherein the hydrogel comprises polyethylene glycol, polypropylene glycol, or copolymers thereof having a molecular weight of at least about 5000 with urethane linkages to polyisocyanates.

33. (previously presented) The biochip of claim 32 wherein each hydrogel cell is between about 20 μm and about 100 μm thick.

34. (currently amended) The biochip of claim 31 wherein each said protein binding entity is directly covalently bound to and within the hydrogel ~~cell~~ cells through reaction with isocyanate groups of said prepolymer that forms said hydrogel.

35. (currently amended) The biochip of claim 31 wherein with said protein binding entities comprise immunoglobins or aptamers.

36. (withdrawn) The biochip of claim 31 wherein each said protein binding entity comprises an enzyme, a receptor, an enzyme inhibitor or an enzyme substrate.

37. (canceled)

38. (withdrawn) The biochip of claim 31 wherein each said protein binding entity is immobilized through a first intermediate agent that is covalently bound to isocyanate groups in the hydrogel and a second intermediate agent linked to said first intermediate agent.

39. (withdrawn) The biochip of claim 38 wherein said first intermediate agent is an antibody and said second intermediate agent is a protein.

40. (withdrawn) The biochip of claim 38 wherein said intermediate agents comprise biotin and avidin.

41. (currently amended) A biochip comprising:

- a) a solid substrate having a flat top surface;
- b) a plurality of optically clear, individual, three-dimensional hydrogel cells at least 20 μm thick comprising urethane polymers of (i) polyethylene glycol, polypropylene glycol, or copolymers thereof and (ii) polyisocyanates, which polymers are isocyanate-functional, and which cells are bound to the flat top surface of said substrate at discrete locations in to form an array of discrete individual three-dimensional cells protruding from said otherwise flat top surface;
- c) intermediate agents immobilized within or upon said hydrogel cells by covalent linkage to reactive isocyanate groups of said hydrogel; and

d) different protein binding entities bound to said intermediate agents within at least several of said hydrogel cells by interaction therewith in a manner so that said protein binding entities can assume their native conformations.

42. (currently amended) The biochip of claim 41 wherein said hydrogel is a urethane-based polymer formed from a prepolymer with excess isocyanate groups in an amount of about 0.2 meq/g to about 0.8 meq/g, and wherein said protein binding entities are bound through pairs of intermediate coupling agents.

43. (previously presented) The biochip of claim 41 wherein said intermediate agent is nitrilotriacetic acid.

44. (withdrawn) A method of carrying out a biochemical assay, which method comprises the steps of:

- (a) providing a biochip according to claim 31 having a plurality of optically clear hydrogel cells bound to a surface of a substrate,
- (b) contacting said hydrogel biochip with an analyte solution potentially containing target biomolecules under binding conditions;
- (c) washing said hydrogel biochip under conditions that remove non-selectively bound and unbound biomolecules; and
- (d) detecting target biomolecules bound to any one of said cells.

45. (withdrawn) The method of claim 44 wherein binding of a target biomolecule results in a compositional change of the protein binding entity in the form of a phosphorylation event or dephosphorylation event.

46. (new) A biochip comprising:

- a) a solid substrate having a flat top surface that is derivatized with groups reactive with isocyanate;
- b) a plurality of optically clear, individual, three-dimensional hydrogel cells at least 20 μm thick bound to the flat surface of the substrate at discrete locations which form an array of discrete individual three-dimensional cells protruding from said otherwise flat top surface, said hydrogel cells being formed from an isocyanate-functional urethane prepolymer wherein up to about 5% of its isocyanate groups are covalently bound to said derivatized groups of said substrate; and
- c) a different binding entity immobilized within or upon various of said hydrogel cells by covalent linkage of said binding entity or an intermediate agent with reactive isocyanate groups of said prepolymer that forms said hydrogel, said entity being effective to selectively hybridize to or sequester a target molecule.